

I CLAIM:

1. A compound having the formula

T-P-M-L

- 5 where T is a labeled species;

M is a metal ion;

P is a peptide or protein that binds the metal ion; and

L is a luminescent label.

- 10 2. The compound of claim 1, where T is an amino acid, a peptide, a protein, a polysaccharide, a nucleotide, an oligonucleotide, a nucleic acid polymer.

3. The compound of claim 1, where T is a drug, a lipid, a lipid assembly, a non-biological organic polymer, or a polymeric microparticle.

- 15 4. The compound of claim 1, where T is a peptide, protein, polysaccharide, nucleotide, oligonucleotide, or nucleic acid polymer.

- 20 5. The compound of claim 1, where L comprises a luminophore that is covalently bound to the metal ion.

6. The compound of claim 5, where L comprises a Eu^{3+} or a Tb^{3+} ion.

7. The compound of claim 5, where the luminophore is a fluorophore.

5 8. The compound of claim 7, where the fluorophore is a xanthene dye.

9. The compound of claim 8, where the xanthene dye is a fluorescein, a rosamine, a rhodamine, or a rhodol.

10 10. The compound of claim 7, where the fluorophore is a benzimidazole, a phenoxazine, an ethidium, a propidium, an acridine, a styryl dye, a carbocyanine, a merocyanines, a coumarin, a pyrene, a chrysene, a stilbene, a carbazine, an anthracene, a naphthalene, a dipyrrometheneboron difluoride, or a dibenzodipyrrometheneboron difluoride.

15 11. The compound of claim 1, where P includes a peptide sequence that incorporates multiple histidine residues and M is a Ni^{2+} ion.

20 12. The compound of claim 1, where P includes a phosphorylated peptide sequence and M is a Ga^{3+} or a Fe^{3+} ion.

13. The compound of claim 1, where T includes a member of a specific binding pair, the compound further comprising a complementary member of the specific binding pair that is associated with T.

5 14. The compound of claim 13, where the complementary specific binding pair member is labeled with a dye that is able to accept luminescence energy transfer from L, or that is able to donate luminescence energy transfer to L.

10 15. The compound of claim 13, where the complex of the complementary specific binding pair member with the compound exhibits detectably different luminescence polarization than that exhibited by the compound alone.

16. The compound of claim 1, where P is chemically conjugated to T.

17. A composition of matter, comprising:

a luminescence energy donor; and

a luminescence energy acceptor;

where the energy acceptor is capable of accepting energy transfer from the energy

5 donor, and where at least one of the energy donor and energy acceptor has the formula

P-M-L

where M is a metal ion;

P is a peptide or protein that binds the metal ion; and

L is a luminescent label.

10 18. The composition of matter of claim 17, where the energy donor and energy acceptor are covalently bound to a molecule.

15 19. The composition of matter of claim 18, where the molecule is susceptible to cleavage by exposure to an appropriate environmental condition, where such cleavage would decrease the degree of energy transfer from the energy donor to the energy acceptor.

20 20. The composition of matter of claim 19, where the appropriate environmental condition comprises the presence of a selected substance.

21. The composition of matter of claim 20, where the selected substance is an appropriate enzyme.

22. The composition of matter of claim 17, where the energy donor is
5 associated with a first member of a specific binding pair, and the energy acceptor is associated with a second member of the specific binding pair, where the first and second specific binding pair members are complementary and are associated with each other such that there is detectable energy transfer from the energy donor to the energy acceptor.

23. The composition of matter of claim 17, where P includes a poly-histidine,
10 M is a Ni^{2+} ion, and L is covalently bound to the Ni^{2+} via a nitrilotriacetic acid chelator.

24. The composition of matter of claim 17, where P includes a phosphorylated peptide, and M is a Ga^{3+} or Fe^{3+} ion.

25. A method of labeling a species, comprising the steps of:

a) conjugating a polypeptide to the species, where the polypeptide is capable of binding a selected metal ion; *not in 1*

b) preparing a label comprising a luminophore that is covalently linked to the selected metal ion; and *not in 1*

c) combining the prepared label with the polypeptide conjugate, under conditions where the polypeptide will bind the selected metal ion.

26. The method of claim 25, where the polypeptide is conjugated to the species via a terminal carboxylic acid group or a terminal amine group.

27. The method of claim 25, where the species is a member of a specific binding pair.

28. The method of claim 27, where the species is a peptide, a protein, an oligonucleotide, or a nucleic acid polymer.

29. The method of claim 25, where the polypeptide comprises a polyhistidine, the luminophore is a fluorescent dye, the selected metal ion is a Ni^{2+} ion, and the fluorescent dye is linked to the metal ion through a nitrilotriacetic acid moiety.

30. A method of detecting energy transfer in a sample, comprising the steps of:

a) contacting the sample with a luminescence energy donor and a luminescence energy acceptor, where the energy acceptor is capable of accepting energy transfer from the energy donor;

5 b) illuminating the sample at a wavelength suitable for excitation of the energy donor; and

c) measuring an amount of luminescence energy transfer from the energy donor to the energy acceptor;

where at least one of the energy donor and energy acceptor has the formula

10 P-M-L

where M is a metal ion;

P is a peptide or protein that binds the metal ion; and

L is a luminescent label.

15 31. The method of claim 30, wherein the step of measuring the amount of luminescence energy transfer from the energy donor to the energy acceptor comprises measuring a decrease in energy donor luminescence.

20 32. The method of claim 30, wherein the step of measuring the amount of luminescence energy transfer from the energy donor to the energy acceptor comprises measuring an increase in energy acceptor luminescence.

33. The method of claim 30, wherein the step of measuring the amount of luminescence energy transfer from the energy donor to the energy acceptor comprises measuring a decrease in energy donor luminescence lifetime.

5 34. The method of claim 30, where the energy donor and the energy acceptor are bound to a first and second member of a specific binding pair, respectively, and further comprising the step of correlating the amount of luminescence energy transfer with the association of the first and second members of the specific binding pair.

10 35. The method of claim 30, where the energy donor and the energy acceptor are bound to first and second portions of the same molecule such that luminescence energy transfer occurs between the energy donor and the energy acceptor, further comprising correlating the amount of luminescence energy transfer with the dissociation of the two portions of the molecule.

36. A method of detecting dissociation of a species, comprising the steps of:

a) providing a species that is labeled with both a luminescence energy donor and a luminescence energy acceptor such that luminescence energy transfer occurs between the energy donor and the energy acceptor;

5 b) exposing the species to a condition that is capable of dissociating the substance such that the energy donor and the energy acceptor are no longer capable of luminescence energy transfer;

c) illuminating the sample at a wavelength suitable for excitation of the energy donor; and

10 d) measuring an amount of luminescence energy transfer from the energy donor to the energy acceptor; and

e) correlating the amount of luminescence energy transfer from the energy donor to the energy acceptor with the dissociation of the species;

where at least one of the energy donor and energy acceptor has the formula

15 P-M-L

where M is a metal ion;

P is a peptide or protein that binds the metal ion; and

L is a luminescent label.

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37. The method of claim 35, where the species is a peptide, a protein, an oligonucleotide, or a nucleic acid polymer.

38. The method of claim 35, where the species is an enzyme substrate, and the
5 step of exposing the species to a condition that is capable of dissociating the species comprises exposing the substance to an appropriate enzyme.

39. A method of detecting a change in conformation of a species, comprising the steps of:

a) providing a species that is labeled with both a luminescence energy donor and a luminescence energy acceptor, where the energy donor is capable of energy transfer
5 to the energy acceptor;

b) illuminating the sample at a wavelength suitable for excitation of the energy donor;

c) measuring a first amount of luminescence energy transfer from the energy donor to the energy acceptor;

d) exposing the species to a condition that is capable of causing the species to change conformation;

e) illuminating the sample at a wavelength suitable for excitation of the energy donor;

f) measuring a change in the luminescence energy transfer from the energy
15 donor to the energy acceptor; and

g) correlating the change in luminescence energy transfer from the energy to the energy acceptor with the change in conformation of the species;

where at least one of the energy donor and energy acceptor has the formula

P-M-L

20 where M is a metal ion;

P is a peptide or protein that binds the metal ion; and

L is a luminescent label.

40. The method of claim 38, where the species is a peptide, a protein, an oligonucleotide, or a nucleic acid polymer.

41. A method of detecting an analyte, comprising the steps of:

5 a) measuring the luminescence polarization of a compound having the formula

T-P-M-L

where T is a member of a specific binding pair;

M is a metal ion;

10 P is a peptide or protein that binds the metal ion; and

L is a luminescent label;

b) contacting the compound with a sample thought to contain the analyte, where the analyte is the complementary member of the specific binding pair;

15 c) incubating the sample for a time sufficient for the compound to form a complex with the analyte;

d) measuring the luminescence polarization of the complex; and

e) correlating the luminescence polarization of the complex with the presence of the analyte.

42. A kit, comprising a compound having the formula

T-P-M-L

where T is a labeled species;

M is a metal ion;

5 P is a peptide or protein that binds the metal ion; and

L is a luminescent label.

43. The kit of claim 41, further comprising at least one additional reagent.

44. The kit of claim 42, wherein the additional reagent is a buffering agent, a
luminescence calibration standard, an enzyme, an enzyme substrate, a nucleic acid stain,
or a labeled antibody.

45. The kit of claim 41, wherein the kit is configured for use in conjunction
15 with a sample in a multiwell microplate or a microfluidic chip.